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EFFECTS OF POLLUTANTS ON EGGS, EMBRYOS AND LARVAE OF AMPHIBIAN SPECIES

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FOR THE COMMANDER

Anthony A. Thomas

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The effects of exposure to pollutants on the development of Rana pipiens and Xenopus laevis embryos and larvae are described. Compounds evaluated include N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, dioctyldiphenylamine, hydrazine sulfate, methylhydrazine, unsymmetrical dimethylhydrazine, and symmetrical dimethylhydrazine. Experiments focused on determination of lethal, teratogenic, and no effect levels of exposure. Techniques for exposing amphibian embryos and larvae to pollutants are also discussed.

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PREFACE

This is the Second Annual Report of work performed under the Environmental Research Addendum P0009 of Air Force Contract AF33615-73-C-4059. This project is titled "Effects of Pollutants on Eggs, Embryos, and Larvae of Amphibian Species."

This report for the period 1 June 1974 to 31 May 1975 contains the results of research efforts concerned with defining the environmental effects of potential environment contamination resulting from the use of certain Air Force materials. This project evaluates the effect of exposure to pollutants on the development of embryos and larvae of frogs. Materials evaluated include N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, dioctyldiphenylamine, hydrazine, methylhydrazine, and dimethylhydrazine. Techniques for exposing amphibian embryos and larvae to these substances are discussed and the results of such exposures are presented.

The author gratefully acknowledges the technical assistance of Ms. Penelope Kimbrell.

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INTRODUCTION AND SUMMARY

This project was initiated as part of a larger study on the effects of potential environmental pollution on aquatic organisms and terrestrial plants resulting from Air Force operations. The objective of this particular phase of the study was to establish a pollutant test system using the egg, embryo, and larval stages of frogs. The system was to be directed at assaying the effects of designated compounds on the development of frogs and to determine ED_{50} and no-effect levels. Initial efforts focused on determination of lethal and teratogenic exposure levels of known teratogens and of compounds specified by the Air Force.

We experimented with two species of frog; Rana pipiens, the Leopard Frog; and Xenopus laevis, the South African Clawed Toad. Both of these species live and breed in aquatic habitats exhibiting a wide range of temperature and varying water quality. They are also sensitive to a number of known teratogenic agents such as trypan blue (Greenhouse and Hamburgh, 1968; Waddington and Perry, 1956), lithium chloride (Hall, 1942), actinomycin D (Flickinger, 1963; Brachet, Denis and De Vitry, 1964), and tetracycline (see results). Preliminary experiments with these species indicated that they were both also sensitive to at least one pollutant supplied by the Air Force (Greenhouse, 1975).

Although preliminary results indicated that both of these species were suitable test organisms we subsequently restricted our studies to Xenopus. This animal offered several advantages as a laboratory species when compared to Rana. These may be summarized as follows: 1) they are easily maintained in a disease-free condition whereas ranid species are very often overcome by bacterial infections when kept in the laboratory; 2) Xenopus will thrive on commercially available trout food whereas Rana must be kept in cold storage or fed live food; 3) each female Xenopus will lay eggs several times per year for a period of several years (some have lived 15 years) in response to commercially available human chorionic gonadotropin, whereas each Rana female can be used only once and requires Rana hormone which must be prepared in the laboratory; and 4) Xenopus embryos metamorphose to froglets in 8-12 weeks whereas Rana pipiens embryos require 6-12 months.

This report describes work performed using the above test system to screen two groups of compounds designated by the Air Force as potential environmental pollutants. The first group consisted of three substituted amines, N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, and p,p'dioctyldiphenylamine. Only N-phenyl-α-naphthylamine was found to have significant toxic effects on developing frog embryos and larvae. The second group of compounds tested included hydrazine and its methylated derivatives monomethylhydrazine and dimethylhydrazine (both symmetrical and unsymmetrical). All four of these compounds were found to have toxic effects on developing frog embryos and/or larvae.

METHODS

Animal Husbandry

Adult Rana pipiens were obtained commercially in the fall and maintained in cold storage at 5 C until needed. When eggs were desired females

were brought to room temperature and induced to ovulate by injection of pituitary extract. Eggs were fertilized by standard procedures (Rugh, 1962).

Adult Xenopus laevis were obtained commercially and maintained in glass aquaria under standard conditions (Brown, 1970). Fertilized eggs were obtained by injecting pairs of frogs with human chorionic gonadotropin by standard laboratory technique (Brown, 1970).

Embryos were grown in aged or dechlorinated tap water in 19 cm inner diameter glass bowls at a density of 100 embryos/liter of water. Later stages were grown in dechlorinated tap water in 30 gallon all glass aquaria at a density of 1 animal/liter. Swimming stage Xenopus are sensitive to handling. When transfer of animals was necessary for counting or observation, they were first anesthetized using ethyl-m-aminobenzoate methane sulfonic acid. This anesthetic has been demonstrated to have no effect on development (McGovern and Rugh, 1944). Transfer was accomplished with a wide mouthed pipet.

R. pipiens embryos were staged according to Shumway (1940). X. laevis embryos were staged according to Nieuwkoop and Faber (1956).

Histology

Specimens were prepared for microscopic examination using the procedures described by Greenhouse and Hamburgh (1968).

Whole mount skeletons were prepared for observation by staining with alizarin red and toluidine blue using a modification of the technique described by Burdi (1965).

MATERIALS

N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine and p,p'dioctyldiphenylamine were supplied by the Aerospace Medical Research Laboratory. Hydrazine was obtained from Sigma Chemical Co. Methylhydrazine and symmetrical dimethylhydrazine were obtained from Aldrich Chemical Co. Unsymmetrical dimethylhydrazine was obtained from Research Organic/Inorganic Chemical Corp.

RESULTS

Control experiments using some known teratogens

In order to demonstrate that frog embryos were susceptible to teratogenesis in our laboratory system, Xenopus embryos were exposed to the known teratogens, trypan blue, lithium chloride, and tetracycline. As expected, exposure to any of these agents did result in aberrant development.

Trypan blue - Commercial trypan blue was purified by chromatography on Sephadex G-25 and then concentrated by flash evaporation. Both R. pipiens and X. laevis embryos are sensitive to 6 hour exposures to this dye. At concentrations of 150 mg/liter, all embryos become malformed if treatment

begins prior to completion of neurulation. Embryos exposed to trypan blue after closure of the neural folds are not affected. The syndrome of malformations exhibited includes microcephaly, tailessness, and reduction of mesonephros (fig. 1).

Lithium Chloride - All embryos exposed to lithium chloride at 100 mg/liter or greater during cleavage and gastrulation exhibited a syndrome of defects affecting the nervous system of Xenopus larvae. The most striking abnormalities included eyelessness and cyclopia (fig. 2).

Tetracycline - Commercially supplied tetracycline supplied as Cosa-Terramycin was teratogenic to all Xenopus embryos at concentrations of 100 mg/liter (fig. 3) when exposure was initiated during cleavage and continued through neurulation.

Effects of N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, and p,p'dioctyldiphenylamine on development of frogs.

The relative insolubility of these amines in water introduced complications not encountered during the earlier control studies which made use of soluble known teratogens. This relative insolubility gave rise to two technical difficulties which significantly affected the interpretation of our data. When added to water these compounds partitioned themselves into a soluble aqueous phase and an insoluble particulate phase. Particles often adhered to embryos and larvae growing in these contaminated environments. If the compound was not toxic this made little difference. However, in the case of N-phenyl-α-naphthylamine which is toxic, embryos and larvae with adhering particles of the amine appeared to suffer more severe damage than did those animals in the same container which did not have particles attached to them. Thus, we were actually working with two populations of animals which led to considerable variability in our results. This problem was partially overcome by filtering our test solutions before adding the animals.

The second difficulty related to the fact that we were never really certain of the actual concentration of the amines in the soluble phase. Regardless of whether the amines were added to the water dry or as acetone solutions some residue always remained. Neither homogenizing the suspensions in a Virtis homogenizer nor shaking them overnight altered this situation. However, homogenized solutions were relatively more toxic than non-homogenized solutions. Toxicity of the N-phenyl-α-naphthylamine also seemed to be related to the surface to volume ratio of our containers. Thus, in one set of containers the minimum concentration of N-phenyl-α-naphthylamine resulting in 100% lethality after a 12 hour exposure was 2 mg/liter. Changing the surface to volume ratio by a factor of 4 increased the minimun concentration causing 100% lethality to 10 mg/liter.

Recently, these difficulties have been circumvented in the following manner. Test solutions were prepared by adding amines to water at a concentration of 2 g/liter. These suspensions were then agitated on a reciprocating shaker for varying intervals of time and then filtered. An aliquot of the filtrate was then extracted with hexane and the concentration of amine in solution was determined by spectrophotometry at 222 nm. By this procedure it was ascertained that dioctyldiphenylamine was practically insoluble in water, that octyl-phenyl-α-naphthylamine had a maximum solubility

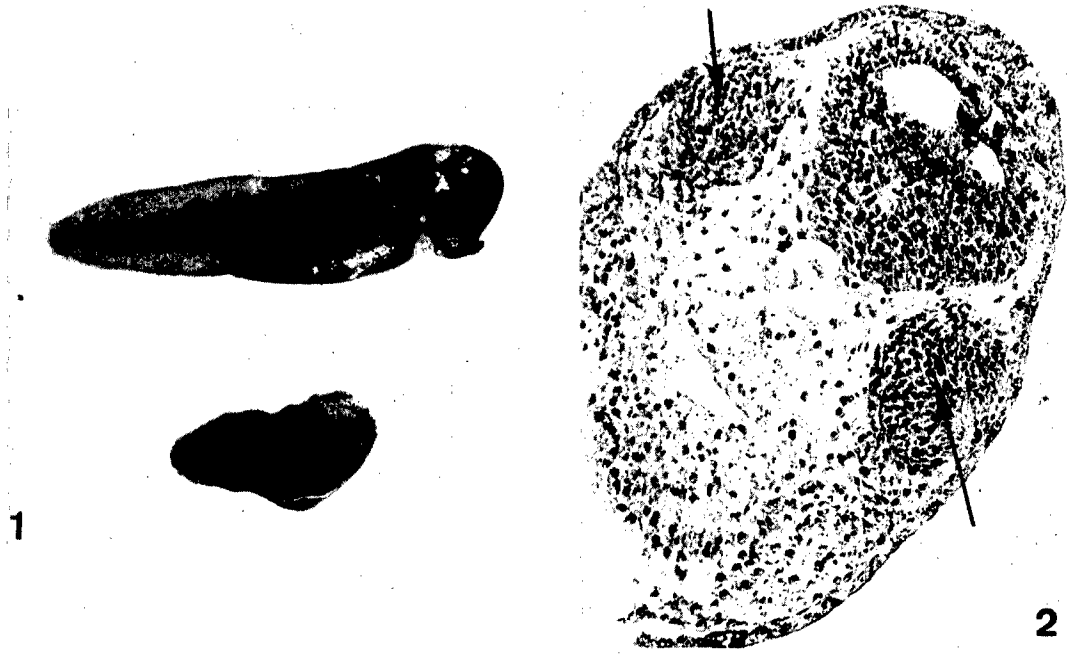


Fig. 1. EFFECT OF TRYPAN BLUE ON DEVELOPMENT OF RANA PIPIENS EMBRYOS. Upper animal is a control. Lower animal is anencephalic due to exposure to trypan blue.

Fig. 2. EFFECT OF LiCl ON DEVELOPMENT OF XENOPUS EMBRYOS. Section through the head of an embryo exposed to LiCl. Note rudimentary eyes (arrows) and abnormal diencephalon.

Fig. 3. EFFECT OF TETRACYCLINE ON DEVELOPMENT OF XENOPUS EMBRYOS. Lower animal is a control.

of approximately 1 mg/liter, and that phenyl-α-naphthylamine had a maximum solubility of approximately 10 mg/liter. The actual concentration of amine in solution was proportional to the length of time for which the suspension was agitated (table 1) and to the temperature of the water (table 2).

N-phenyl-α-naphthylamine - This was the only one of the three amines which was toxic to frog embryos and larvae in concentrations tested. Exposure to this amine produced abnormal development and/or death. The actual response of the test animals varied with the concentration of the amine and was also dependent upon the duration of exposure and the developmental stage of the test animals.

Eggs and embryos of Rana pipiens or Xenopus laevis show no effects from exposure to N-phenyl-α-naphthylamine until they reach stages 18 and 24 respectively. These stages are characterized by motor reactions to external stimuli. Embryos exposed to this compound usually fail to respond to external pricking. Xenopus embryos fail to develop further if kept in contact with the amine. Rana embryos develop to stage 20 characterized by beating heart and blood circulation in external gills, at which point development is arrested. Continued exposure leads to death (table 3).

Exposure to N-phenyl-α-naphthylamine was also toxic to later stage larvae of both species. When exposed to lethal concentrations the following syndrome was observed. During the first hour of exposure swimming activity decreases. Swimming movements cease by the end of the second hour, but larvae are still capable of movement in response to external stimuli. Heart beat gradually slows and becomes irregular. Death occurs within 24-48 hours of continuous exposure (table 4).

Lethal concentrations of this compound varied from 200 mg/liter to as little as 2 mg/liter depending upon how the suspension was prepared and the final surface to volume ratio of the containers.

Exposure of Xenopus larvae to concentrations of N-phenyl-α-naphthylamine $\leq$5.6 mg/liter had no observable effect on viability. Concentrations $\geq$6.0 mg/liter were lethal (table 5). Larvae growing in lethal concentrations of this compound, but transferred to fresh uncontaminated water after only brief exposures, survived and usually developed normally. Survival rate was inversely proportional to duration of exposure (tables 6a and 6b).

Exposure of embryos to sublethal concentrations of N-phenyl-α-naphthylamine results in retardation of growth and abnormal development. Exposure of Xenopus embryos to concentrations $\geq$5.2 mg/liter was teratogenic (table 7). Figures 4 and 5 illustrate the teratogenic effect on Xenopus embryos. Most malformed embryos die before or during metamorphosis. The syndrome of anomalies is complex affecting the head and trunk. Neither limbs nor tail are affected.

Octyl-phenyl-α-naphthylamine and p,p'dioctyldiphenylamine - Neither of these compounds was toxic to embryos or larvae of either species in concentrations of up to 1 g/liter. High concentrations of octyl-phenyl-α-naphthylamine may retard the growth rate of tadpoles. However, histological study indicated that these animals were not malformed. They metamorphosed into apparently normal froglets (tables 3,4,5 & 7).

Table 1

SOLUBILIZATION OF AMINES AS A FUNCTION OF TIME

Time on Shaker	mg/liter in Solution
N-phenyl-α-naphthylamine	
24	3.64
72	5.04
96	5.56
Octyl-phenyl-α-naphthylamine	
24	0.44
96	1.00

Suspensions of amine were prepared by adding 1g of either N-phenyl-α-naphthylamine or octyl-phenyl-α-naphthylamine to 500 ml of water. The suspensions were agitated on a reciprocating shaker at room temperature and aliquots were removed at intervals. The aliquots were filtered and then extracted with hexane. The concentrations of amine was then determined by spectrophotometry.

Table 2

SOLUBILIZATION OF N-PHENYL-α-NAPHTHYLAMINE AS A FUNCTION OF TEMPERATURE

Temperature ^{o}C	mg/liter in Solution
5	1.92
17	2.76
35	5.20

Suspensions of N-phenyl-α-naphthylamine were prepared by adding 100g of the amine to 100 ml of water. The suspensions were agitated for 24 hours at the above temperatures and the concentration of amine in solution was then determined as in Table 1.

Table 3

EFFECT OF CONTINUOUS EXPOSURE OF RANA PIPIENS EMBRYOS TO AMINES

Treatment	Number of Embryos Exposed	Number of Embryos Surviving		
		Stage 10 (Dorsal Lip)	Stage 18 (Muscular Response)	Stage 20 (Heart Beat)
Control	100	100	97	90
N-phenyl-α-naphthylamine				
20 mg/liter	100	100	0	0
200 mg/liter	100	100	0	0
Octyl-phenyl-α-naphthylamine				
20 mg/liter	100	100	95	92
200 mg/liter	100	100	90	88
Dioctyldiphenylamine				
20 mg/liter	100	100	91	85
200 mg/liter	100	100	90	80

Table 4

EFFECT OF EXPOSURE OF RANA PIPIENS LARVAE TO AMINES

Treatment	No. of Larvae Exposed	No. of Larvae Surviving 24 Hrs.	No. of Larvae Surviving 48 Hrs.
Control	125	125	125
N-phenyl-α-naphthylamine			
5 mg/liter	125	125	0
Octyl-phenyl-α-naphthylamine			
50 mg/liter	125	125	125
Dioctyldiphenylamine			
100 mg/liter	125	125	125

Table 5

EFFECT OF EXPOSURE TO SOLUTIONS OF NAPHTHYLAMINES ON VIABILITY OF XENOPUS LARVAE

Compound	Conc. mg/liter	No. Larvae Exp.	No. Larvae Surv. 124 hr.	168 hr.
Control	0.0	90	84	82
N-phenyl-α-naphthylamine	5.6	85	85	80
	6.0	75	75	0
	6.2	20	0	0
	7.1	36	0	0
N-phenyl-α-naphthylamine Tris buffer pH 7.6	6.0	75	75	0
Octyl-phenyl-α-naphthylamine	0.67	46	46	43
	1.00	15	15	15

Table 6a

EFFECT OF VARYING LENGTHS OF EXPOSURE TO N-PHENYL-α-NAPHTHYLAMINE ON THE SURVIVAL OF XENOPUS LARVAE I.

Exposure Time	Number of Larvae	Survival Time 24 Hours	30 Days
Control	50	50	43
12 Hours	50	41	41
24 Hours	50	11	11

Table 6b

EFFECT OF VARYING LENGTHS OF EXPOSURE TO N-PHENYL-α-NAPHTHYLAMINE ON THE SURVIVAL OF XENOPUS LARVAE II.

Exposure Time	Number of Larvae	Survival Time 24 hrs.	5 days	12 days	20 days
Control	100	100	97	97	95
24 Hours	100	100	98	86	81
30 Hours	100	100	13	13	13
36 Hours	100	100	7	7	0
4 Days	100	100	22	19	2

Xenopus larvae were cultured in a saturated solution of amine for the stated intervals and then removed and placed in fresh uncontaminated tap water. The saturated solution was prepared by adding N-phenyl-α-naphthylamine to water at a concentration of 100 mg/liter. This suspension was mixed for 48 hrs. on a reciprocating shaker and then filtered before use. The large difference in survivability at 24 hrs. between tables 6a & 6b is not easily explained. However, in comparing these tables three sources of variability must be considered: 1) The two experiments made use of different batches of larvae, 2) the larvae were of different stages, and 3) the solubility of the N-phenyl-α-naphthylamine varied with laboratory conditions.

Table 7

EFFECT OF EXPOSURE TO SOLUTIONS OF OCTYL-PHENYL-α-NAPHTHYLAMINE AND N-PHENYL-α-NAPHTHYLAMINE ON XENOPUS EMBRYOS

Compound	Conc. mg/liter	No. Emb. Exp.	No. Emb. Malf.	% Surv. Malf.	No. Emb. Died
Control	0.0	100	1	1.4	28
Octyl-phenyl-α-naphthylamine	1.0	100	8	8.9	10
N-phenyl-α-naphthylamine	5.2	100	17	23.3	27
	6.2	100	21	100	79
	7.2	100	10	100	90

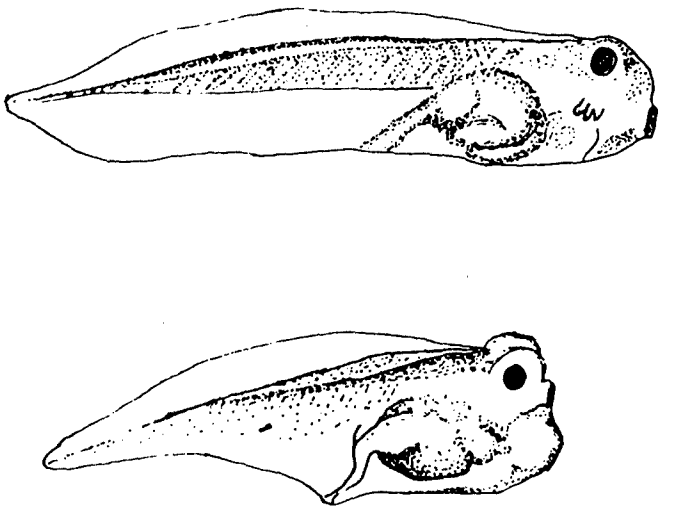


Fig. 4. EFFECT OF N-PHENYL-α-NAPHTHYLAMINE ON DEVELOPMENT OF XENOPUS LARVAE.

Camera lucida drawing of control larva (above) and larva which was exposed to N-phenyl-α-naphthylamine for 72 hours commencing at stage 9 (blastula).

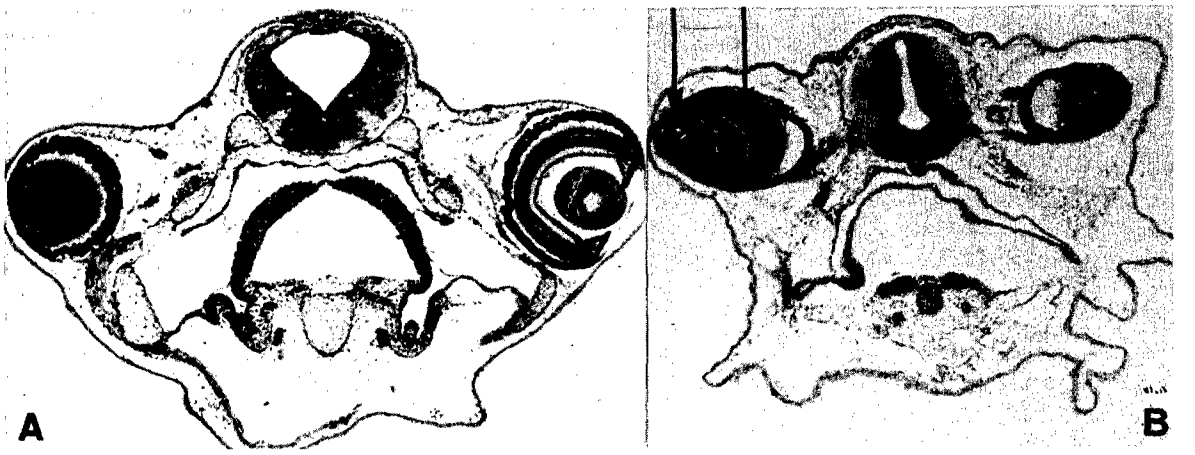


Fig. 5. EFFECT OF EXPOSURE TO N-PHENYL-α-NAPHTHYLAMINE ON EYE DEVELOPMENT.

A-Control, B-Experimental. Note retarded development of the retina and abnormal position of the lens in the experimental embryo.

Effects of hydrazine sulfate, methylhydrazine, symmetrical dimethylhydrazine, and unsymmetrical dimethylhydrazine on development of Xenopus laevis

These compounds are soluble in water and therefore did not present the technical difficulties incurred in studies with the organic amines. However, they do degrade in water at varying rates. Therefore, the concentrations stated in the text are only valid as initial concentrations. Solutions were changed twice weekly.

Hydrazine sulfate - This compound was not found to be toxic to post-hatching larvae (stage 35) in concentrations up to 400 mg/liter (table 8). We have no evidence to indicate that hydrazine sulfate interferes with limb development or metamorphosis at this concentration. Higher concentrations have not been tested.

Hydrazine sulfate is teratogenic at concentrations of 40 mg/liter or higher if exposure occurs prior to the completion of neurulation (stage 22-23). Data are summarized in table 9. The syndrome of malformations produced by exposure to this compound includes foreshortening of the axial skeleton, tail kinks and edema (fig. 6). The primary effect seems to be an inhibition of the normal elongation of the embryo following closure of the neural folds. This is followed by edema and formation of cysts between the ectoderm and mesoderm. These cause kinks of the tail and some secondary displacement of internal organs which are otherwise normal in appearance.

Brief exposures of embryos to hydrazine sulfate are not in themselves lethal, even at concentrations which lead to teratogenesis. Malformed embryos will die if left in contact with hydrazine sulfate. However, malformed embryos which are transferred to fresh uncontaminated water usually survive and in time appear normal. At this time we have no data on ability of these "rescued" larvae to metamorphose.

Monomethylhydrazine - Studies with this compound are still in progress. Preliminary data are summarized in table 10. Continuous exposure of embryos (beginning at blastula) to $\leq$1 mg/liter of methylhydrazine appears to have no effect on viability or development. At concentrations of 10 mg/liter or greater this compound is lethal to embryos. We have no data concerning exposure of larval stages.

Symmetrical Dimethylhydrazine - This compound has toxic effects on Xenopus embryos and larvae. Continuous exposure of embryos or larvae to $\leq$10 mg/liter appears to have no toxic effects. Continuous exposure of embryos (beginning at blastula) to 100 mg/liter of this compound is toxic to 100% of the animals. After two weeks of exposure, 50% of the embryos are dead. The remaining 50% exhibit tail malformations. These data are summarized in tables 11 & 12. Screening studies are still in progress.

Unsymmetrical Dimethylhydrazine - This compound is toxic to Xenopus embryos. Concentrations up to 1 mg/liter are neither lethal nor teratogenic. Ten mg/liter of this compound is highly teratogenic to all embryonic stages whereas 100 mg/liter is lethal. These data are summarized in tables 13 & 14. The most common malformations observed include foreshortening of the body and tail, kinks of the tail, and edema. A significant number of embryos that were exposed to this compound also exhibit microcephaly. Occasionally embryos with two tails were observed (fig. 7-10).

Table 8

EFFECT OF HYDRAZINE SULFATE ON VIABILITY OF XENOPUS LARVAE (Stage 35)

Hydrazine Concentrations mg/liter	No. of Larvae Initially Exposed	No. of Larvae Malformed after 7 Days Exposure	No. of Larvae Dead after 7 Days Exposure
Control	100	0	0
0.4	100	0	3
4	100	0	2
40	100	1	1
400	100	3	4

Table 9

EFFECT OF CONTINUOUS EXPOSURE TO HYDRAZINE SULFATE ON DEVELOPMENT OF XENOPUS EMBRYOS

Hydrazine Concentrations mg/liter	Stage at Initial Exposure	No. Embryos Exposed	No. Embryos Malformed after 5 days
Control	Blastula	200	0
40	Blastula	200	200
400	Blastula	200	200

Table 10

EFFECT OF CONTINUOUS EXPOSURE TO (MONO)METHYLHYDRAZINE ON DEVELOPMENT OF XENOPUS EMBRYOS

	Number Embryos Exposed	Number Embryos Malformed After 3 Days	% Malformed	Number Embryos Dead after 3 Days
Control	300	12	4	21
1 mg/liter	100	4	4	5
10 mg/liter	200	0	0	200
100 mg/liter	100	0	0	100

Table 11

EFFECT OF CONTINUOUS EXPOSURE TO SYMMETRICAL DIMETHYLHYDRAZINE ON XENOPUS EMBRYOS

	Stage at which Exposure was Initiated	No. Embryos Exposed	No. Embryos Malformed after 15 Days	No. Embryos Dead after 15 Days
Control		100	7	6
0.1 mg/liter	7	100	4	10
1 mg/liter	7	100	2	8
10 mg/liter	7	100	5	10
100 mg/liter	7	100	49	48

Table 12

EFFECT OF SYMMETRICAL DIMETHYLHYDRAZINE ON XENOPUS LARVAE (stage 35)

	No. Embryos Exposed	No. Embryos Malformed after 15 days	No. Embryos Dead after 10 Days
Control	500	0	30
0.1 mg/liter	100	0	0
1 mg/liter	100	0	50
10 mg/liter	500	0	2
100 mg/liter	100	0	100

Table 13

EFFECT OF CONTINUOUS EXPOSURE TO UNSYMMETRICAL DIMETHYLHYDRAZINE ON XENOPUS EMBRYOS

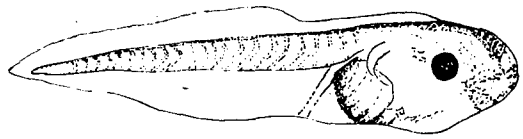
	Stage at which Exposure was Initiated	No. Embryos Exposed	No. Embryos Malformed after 9 Days	No. Embryos Dead after 9 Days
Control		100	0	7
0.1 mg/liter	7	100	2	10
1 mg/liter	7	100	3	11
10 mg/liter	7	100	86	10
100 mg/liter	7	100	0	100

Table 14

EFFECT OF EXPOSURE TO UNSYMMETRICAL DIMETHYLHYDRAZINE ON XENOPUS EMBRYOS AT DIFFERENT STAGES

Stage at which Exposure was begun		Number of Embryos	Number of Embryos Malformed after 10 Days	% Embryos Malformed	Number Embryos Dead
Blastula (stage 7-9)	Controls	300	10*	3	24
	Exptls.	500	207	41	24
Gastrula (stage 10-12	Controls	400	1	0.25	2
	Exptls.	400	251	63	2
Neural Plate (stage 13)	Controls	500	2	0.4	34
	Exptls.	500	90	18	33
Tail Bud (stage 26-28)	Controls	400	1	0.25	54
	Exptls.	400	279	70	112

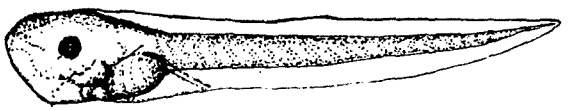
*The only abnormality observed in control embryos was edema.



CONTROL

EXPERIMENTAL

Fig. 6. EFFECT OF HYDRAZINE SULFATE ON DEVELOPMENT OF XENOPUS.
Camera lucida drawing of control (above) and experimental (below) which was exposed to 100 mg/liter hydrazine continuously commencing at gastrula stage. Note microphthalmia and micromvelia.



CONTROL

20

EXPERIMENTAL

Fig. 7. EFFECT OF UNSYMMETRICAL DIMETHYLHYDRAZINE ON DEVELOPMENT OF XENOPUS EMBRYOS.

Camera lucida drawing of a control larva and a larva exposed to UDMH for 15 days commencing at gastrula.

Fig. 8. ANENCEPHALY CAUSED BY EXPOSURE OF XENOPUS EMBRYOS TO UNSYMMETRICAL DIMETHYLHYDRAZINE.

Larva on left is a control.

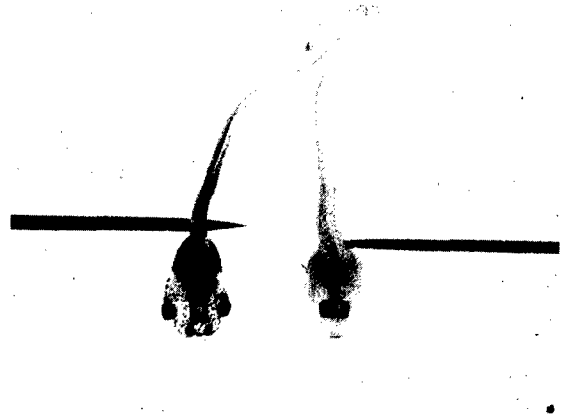


Fig. 9. REDUCTION OF FOREBRAIN CAUSED BY EXPOSURE OF XENOPUS EMBRYOS TO UNSYMMETRICAL DIMETHYLHYDRAZINE.
Larva on the left is a control.

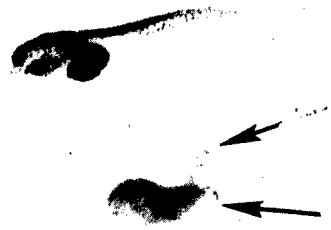


Fig. 10. DUPLICATION OF THE TAIL (arrows) CAUSED BY EXPOSURE OF XENOPUS EMBRYOS TO UNSYMMETRICAL DIMETHYLHYDRAZINE.

Experiments are in progress to study the effects of this compound on larval stages.

COMMENTS AND RECOMMENDATIONS

The data presented concerning effects of compounds on embryonic development is more complete than the data presented concerning the effect of these same compounds on development of larval stages. This is due to the sensitivity of larval stages to handling. Growth of larvae is also affected by population density and the frequency with which water is changed. We have now worked out methods for raising large numbers of larvae under conditions which allow for uniform and rapid growth and low mortality. This will allow us to screen compounds more effectively and to measure growth rates in addition to viability and teratogenicity.

A number of the compounds screened during these studies induce malformations in developing frogs. Two of the compounds, N-phenyl-α-naphthylamine and unsymmetrical dimethylhydrazine, induce severe abnormalities of the central nervous system including microcephaly, micromyelia, and microphthalmia. Mammalian (including human) embryos and fetuses also exhibit these congenital anomalies. It would appear, therefore, that these compounds would be prime candidates for teratogenic testing in mammalian systems to better define the health hazard associated with these compounds.

GLOSSARY

Anencephaly	-	Absence of the cerebral and cerebellar hemispheres, with only a rudimentary brain stem.
Embryo	-	The developing frog is an embryo from fertilization until feeding begins. This period is characterized by organogenesis with little or no change in mass.
Larva	-	Once the embryo has emerged from its external membranes (hatched) and commences feeding, it is a larva. By the time an embryo becomes a larva the main organ rudiments are present. This stage is characterized by rapid growth.
Microcephaly	-	Abnormal smallness of the head and brain.
Micromyelia	-	Abnormal shortness of the spinal cord.
Microphthalmia	-	Abnormal smallness of the eyes.

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